Al-malki et al., Afr J Tradit Complement Altern Med. (2017) 14(2):234-240
doi:10.21010/ajtcam.v14i2.25

SIGNALING PATHWAYS REGULATED BY *BRASSICACEAE* EXTRACT INHIBIT THE FORMATION OF ADVANCED GLYCATED END PRODUCTS IN RAT BRAIN.

Abdulrahman L. Al-Malki[1,2,3],Elie K. Barbour[3,4], Huwait EA[1,2,5] Said S. Moselhy[1,2,3,6] Anas Hassan Saeed ALZahrani [7]and Taha A. Kumosani[1,3,8].

Department of Biochemistry, Faculty of Science, King Abdulaziz University (KAU), P.O Box 80203, Jeddah, Saudi Arabia. [2]Bioactive Natural Products Research Group, KAU.[3] Experimental Biochemistry Unit, King Fahd Medical Research Center, KAU. [4]Agriculture Department, Faculty of Agricultural and Food Sciences, American University of Beirut, Beirut, Lebanon; Adjuncted to Biochemistry Department, Faculty of Science, KAU. [5]Vitamin D Pharmacogenomics Research Group, King Abdulaziz University. [6]Department of Biochemistry, Faculty of Science, Ain Shams University. [7]Medical Student,5th year, King Abdulaziz University. [8] Production of bioproducts for industrial applications Research Group, KAU.

Corresponding author Email: alalmalki@kau.edu.sa

Abstract

Background: The goal of this study was identification signaling molecules mediated the formation of AGEs in brain of rats injected with $CdCl_2$ and the role of camel whey proteins and *Brassicaceae* extract on formation of AGEs in brain. **Methods**: Ninety male rats were randomly grouped into five groups; Normal control (GpI) and the other rats (groups II-V) were received a single dose of cadmium chloride *i.p* (5 µg/kg/b.w) for induction of neurodegeneration. Rats in groups III-V were treated daily with whey protein (1g/kg b.w) or *Brassicaceae* extract (1mg/kg b.w) or combined respectively for 12 weeks.
Results: It was found that whey protein combined with *Brassicaceae* extract prevented the formation of AGEs and enhance the antioxidant activity compared with untreated group ($p < 0.001$). Serum tumor necrosis factor (TNF-α) and interleukine (IL-6) levels were significantly decreased ($p<0.01$) in rats treated with whey protein and *Brassicaceae* extract formation compared with untreated. The combined treatment showed a better impact than individual ones ($p<0.001$)**.** The level of cAMP but not cGMP were lowered in combined treatment than individual ($p<0.01$). C**onclusion,** it can be postulated that Whey protein + *Brassicaceae* extract formation could have potential benefits in the prevention of the onset and progression of neuropathy in patients.

Keywords: Whey protein- *Brassicaceae* extract -neurodegeneration -rats

Introduction

Signal transduction is involved in cell function [Agrawal etal., 2005], development [etal.,2003], differentiation [Alberti etal.,1998], apoptosis and cell death. Signaling molecules including hormones, neurotransmitters and growth factors involve binding to target receptors initiates a process of cascade and finally response [AL-Lawati etal.,2002]. The amplified signal is then propagated to the nucleus, resulting in induction or repression of gene expression [Ashour etal.,1999]. Oxidative damage are often related with path physiology of many diseases [Badr etal.,2011]. Signaling pathways including protein dependent kinases (PK) and secondary messenger as cAMP, cGMP, IP3 and calcium. Identification of these mediators are important rational of identification of pathogenesis and therapeutic protocol [Beg etal.,1986]

Cadmium (Cd) is a toxic heavy metal, considered as a human carcinogen by National Toxicology Agency [Floweler ,2009]. Cadmium (Cd) has a long half-life due to its low rate of elimination from the body. Thus, long-term exposure to Cd will lead to toxicity due to its accumulation in different tissues as brains and central nervous system (CNS). Cd can be taken by inhalation and cross the blood brain barrier (BBB) permeability [Satarug etal., 2010]. The previous study indicated the hepatic-toxic effect of Cd that was associated with oxidative stress and tissue damage.
The primary neurodegenerative diseases lead to dementia and loss of concentration [Renugadevj and Miltonprabu,2009] In neurodegenerative disease, the early stages, characterized by his inability to acquire new memories and difficulty in recalling When late stage, it is diagnosed with behavior assessments**.** The symptoms of late stage include confusion, mood swings, language breakdown, long-term memory loss, [Eldemerdash etal.,2004]. loss of body functions, leading to death.

Although the mechanism of Cadmium is not yet been well documented as a result of the pathophysiology of Cadmium, several factors are reported to play a role as activation of NF-κB [Ebaid etal.,2005]. Activation of the renin–angiotensin system, acceleration of oxidative stress, activation of specific kinase and subsequent formation of advanced glycation end products (AGEs) are major risk factors. As a result of the injection with $CdCl_2$, increased overproduction

doi:10.21010/ajtcam.v14i2.25
reactive oxygen species (ROS). These ROS results in the glycation of proteins which enhance the formation of AGE which accumulated in different tissues causing its damage.Previous studies reported that camel milk have beneficial role in Cadmium toxicity and many other diseases. Therefore, using of camel milk is a useful addition to the insulin used for the treatment of type I diabetes [Eeg etal.,2008].Whey protein is believed to be the best available protein, when compared to other proteins . Due to its regulatory effect on the key enzymes that regulate glucose and glycogen metabolism. In addition to the fact that, whey protein contains all amino acids that are necessary for cell growth [Floyed etal.,1970]. *Brassicaceae* extract was traditionally used in treatment of colic disease, diarrhea, sedative (13) .

The potential anti oxidant activity of *Brassicaceae* extract are due to their content of phenolic compounds (14). It was reported that water extracts of functional foods proved to have a potent antioxidant function. It was proved that, a correlation between serum levels of *Brassicaceae* extract and metabolic disease as diabetes. The signaling pathway is missing for this mechanism.

The aim of this study was identification of signaling molecules including secondary messenger as cAMP, cGMP. Secondly, evaluate the role of camel whey proteins and *Brassicaceae* extract on the signals mediated the formation of AGEs in CdCL2 induced neurodegeneration and the mechanism of its action in an experimental rat model.

Materials and Methods

Nine week-old (120±20g) male Albino rats (n=90) will be obtained from KFMRC, King Abdulaziz University (Jeddah). The animals will be housed in cages and foods and tap water were given *ad libitum*. Rats will be randomly grouped into control (Group I) (n=15), received a single dose of 0.1 mol/L citrate buffer. And the other rats (groups 2-5, 75 rats) were received a single dose of injected with $CdCl_2$ *i.p*, at dose of 5 µg/kg. Rats in group II were considered diabetic untreated. Rats in groups III-V were treated daily with whey protein (1g/ kg b.w) or *Brassicaceae* extract (1 mg/kg b.w) or combined for 6 weeks. Animals were fasted overnight; blood was collected directly from the heart. Serum was used for the determination of glucose, glycated hemoglobin, advanced glycated end products (AGES), malondialdhyde, superoxide dismutase, total antioxidant activity, urea and creatinine, inflammatory cytokines as tumor necrosis factor (TNF-α) and interleukine (IL-6) levels will be determined by using ELISA kit .

Biochemical analysis in brain tissues

Brain tissue (0.1g) was homogenized in 2 ml phosphate buffer (pH 7.3) contain protease inhibitor. Samples were centrifuged at 12,000 rpm/10 minutes at 5°C. The filtrate was used for the determination of malondialldhyde (MDA), reduced glutathione (GSH), catalase, superoxide dismutase by using commercial kits from BIORAD (England), tumor necrosis factor, interleukine-1, and advanced glycated end products (AGEs) levels by using ELISA kits. Protein levels was determined by Foline reagent using a standard curve [Joseph etal.,1999]. The levels of IL-6 and TNF-α were determined by ELISA using the commercially available kits according to the instructions of the manufacturer. Signaling molecules including secondary messenger as cAMP, cGMP were determined.

Assay of cAMP and cGMP.

Guanosine 3,5-cyclic monophosphate (cGMP) and cAMP in tissues were determined by direct immunoassay Kit with sensitivity, 1~100 fmol /ug protein per assay, than the cGMP Direct Immunoassay Kit, from antibody Biovision company. The level expressed as fmol of cAMP while for cGMP as pmol quantities.

Statistical analysis

Statistical analysis will be done by SPSS using student's *t*-test. Value of $P < 0.05$ was considered as statistically significant. ANOVA test will be performed to correlate between different groups.

Results

Administration of rats with $CdCl_2$ resulted in a significant increase in blood glucose levels in the diabetic group compared with the control group ($p<0.001$), while treatment with whey protein or *Brassicaceae* extract or combined resulted in a significance decrease in blood glucose compared with the untreated diabetic animals ($p<0.05$, table 1). As a result of diabetes, HbA1c was significantly increased ($p<0.05$) in the diabetic untreated group. Treatment of animals with Whey protein + *Brassicaceae* extract improves the two parameters (table 1). The Whey protein + *Brassicaceae* extract exert hypoglycemic action but less potent than insulin ($p<0.05$). The antioxidant activities GSH reduced, catalase, SOD in brain of diabetic animals were significantly reduced as a result of $CdCl_2$ injection. Supplementation of whey protein or *Brassicaceae* extract or combination resulted in a significant elevation in GSH

doi:10.21010/ajtcam.v14i2.25

(p<0.05 for each) level and the activities of catalase and SOD (p<0.001, <0.01 and <0.05) respectively (table 1). Administration of combination resulted in better decrease of MDA levels than individual ones (table 2).

Figures (1, 2) showed that, serum aldolase reductase and total antioxidant activities were significantly decreased in diabetic arts compared with control ones (p<0.01 and 0.001) respectively. Whey protein or *Brassicaceae* extract or combined showed an elevation in these activities with dose dependent. The enhancement in insulin injection is better than combination (p<0.05).

Fig.3 showed that, both TNFα and IL-1 play an important role in the pathogenesis of diabetic neuropathy. As a result of CdCl2 injection the brain levels of TNF-α and IL-1 were significantly elevated (p<0.001) for each indicating a considerable level of inflammation compared with the normal control rats. Administration of whey protein or *Brassicaceae* extract or combined results in a significant reduction of these elevated levels (p<0.01. Whey protein + *Brassicaceae* extract supplementation resulted in a significant reduction of AGEs in a dose dependent manner as indicated in Figure3. Combination effect attenuated AGE production compared with individual treatment (p<0.001).

Table 1: body weight, glucose and HbA1c levels in different groups.

	Group 1	Group II	Group III	Group IV	Group V
Initial body weight(gm)	120.13±9.5	125.44±7	129.19±6	122±7.2	131.33±6.5
Final body weight(gm)	199.5±8.2	150.8±7.2[a]	167.3±16.5[a,b]	165.52±8.9[a,b]	210±12.3[a,b]
Glucose (mg/dl)	92.78±0.45	275.35±1.45[a]	145.92±1.4[a,b]	137.71±1.23[b]	102.21±0.57[a,b]
HbA1c(%)	5.54±0.41	8.42±0.34[a]	6.8±0.54 [b]	6.8±0.38 [b]	5. 8±0.52 [b]

(a): *p* value significant versus control.
(b) *p* value significant versus diabetic.

Table 2: Serum and brain malondialdhyde (MDA) level and reduced glutathione (GSH), catalase, and superoxide dismutase (SOD) activity in brain of different groups (Mean ±S.D)

Groups	Serum MDA (µ mol /L)	Brain			
		MDA µmol/mg protein	GSH µg /mg protein	Catalase U/mg protein	SOD U/mg protein
Group I (n = 15) Range Mean ±S.D.	 24 - 15.6 75 ± 12	 98-196 66.7 ± 28	 104 – 546 284 ±52	 992-2349 1249 ±140	 956 – 2623 1545 ±191
Group II (n= 12) Range Mean ±S.E. *p** value	 40- 89 48 ± 5.9 < 0.01	 190-261 188 ± 37 N. S.	 68.7-221.5 123.6 ±19.3 < 0.01	 109.5 – 642 358 ±65 <0.001	 453-1342 760±78 <0.001
Group III (n = 13) Range Mean ± S.E. *p** value	 33 –56 31±12.2 < 0.01	 149 – 213 142 ± 32 <0.001	 121 – 496 259 ±50 <0.001	 706 – 2002 843 ±134 <0.001	 876 -1987 1021 ±163 <0.001
Group IV (n= 13) Range Mean ±S.E. *p*** value	 16 – 73.4 33 ±2.7 < 0.001	 136-261 133± 25 <0.001	 69.6- 213.9 128±16.4 p< 0.05	 456 -1632 987 ±56 <0.001	 962-1316 836±53 <0.001
Group V (n= 14) Range Mean ±S.E. *p*** value	22 – 73.4 38 ±6.7 < 0.001	 156-261 121± 25 N.S.	 69.6- 213.9 128 ± 16.4 p< 0.05	 546 -1756 1112 ±86 <0.001	 624-2116 1233±153 <0.001

*P** value, all groups vs control
*P*** value, treated Vs untreated

doi:10.21010/ajtcam.v14i2.25

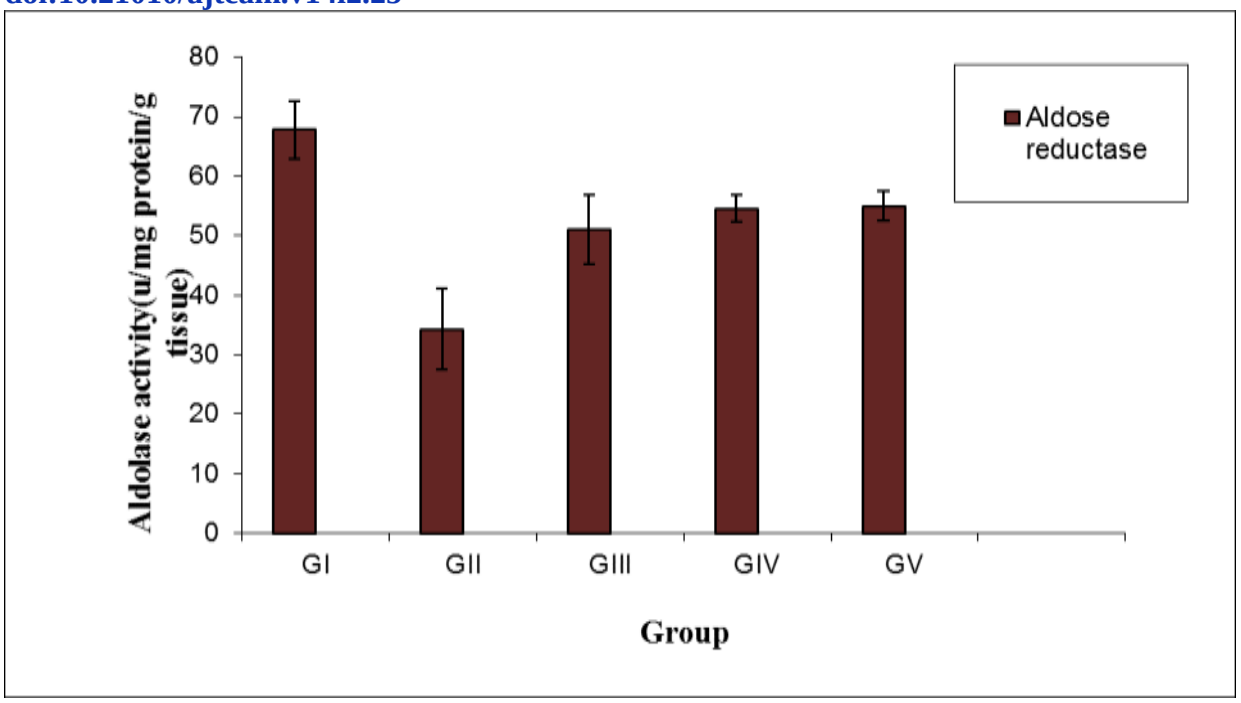

Figure 1: Serum aldose reductase activity in all studied groups (Mean ±SD).

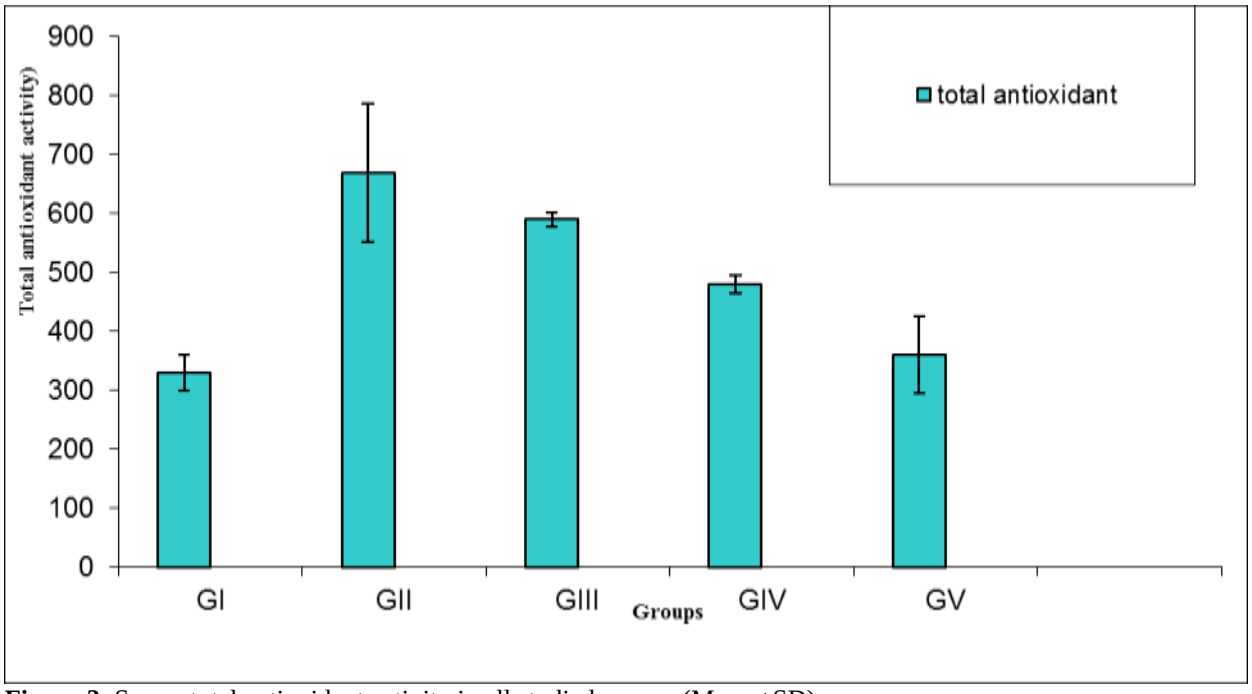

Figure 2: Serum total antioxidant activity in all studied groups (Mean ±SD).

doi:10.21010/ajtcam.v14i2.25

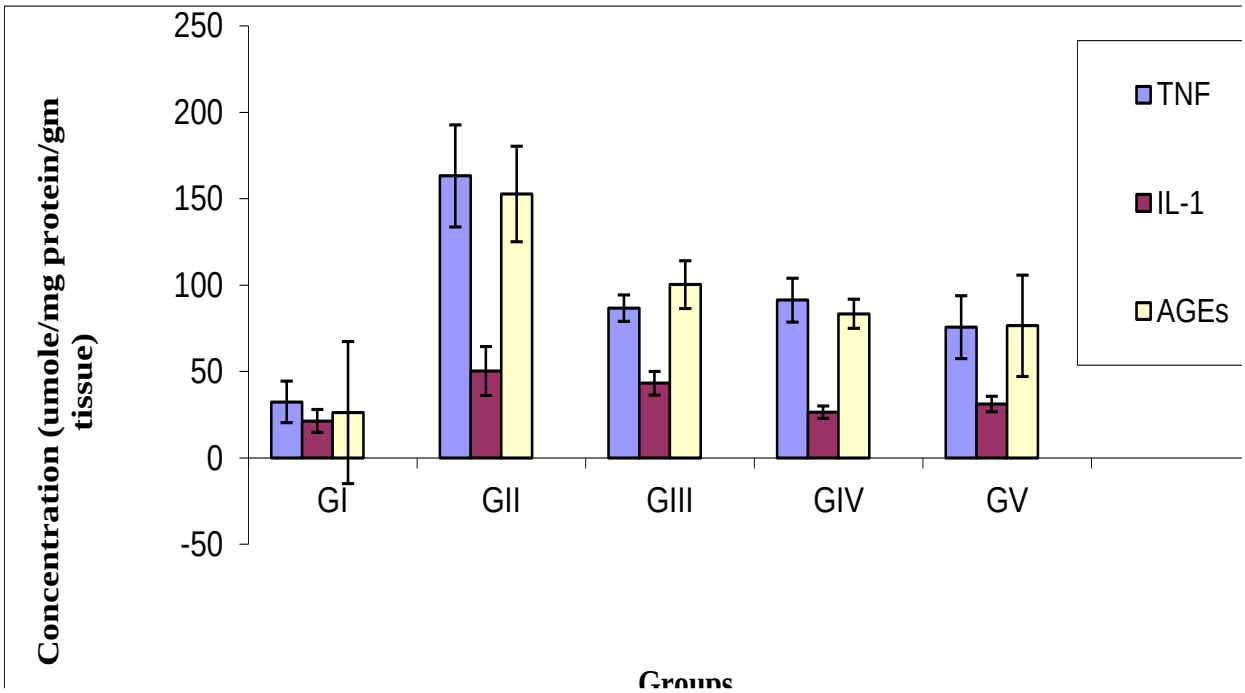

Figure 3: Brain tumor necrosis factor (TNF- α), interleukine-1, and advanced glycated end products (AGEs) levels

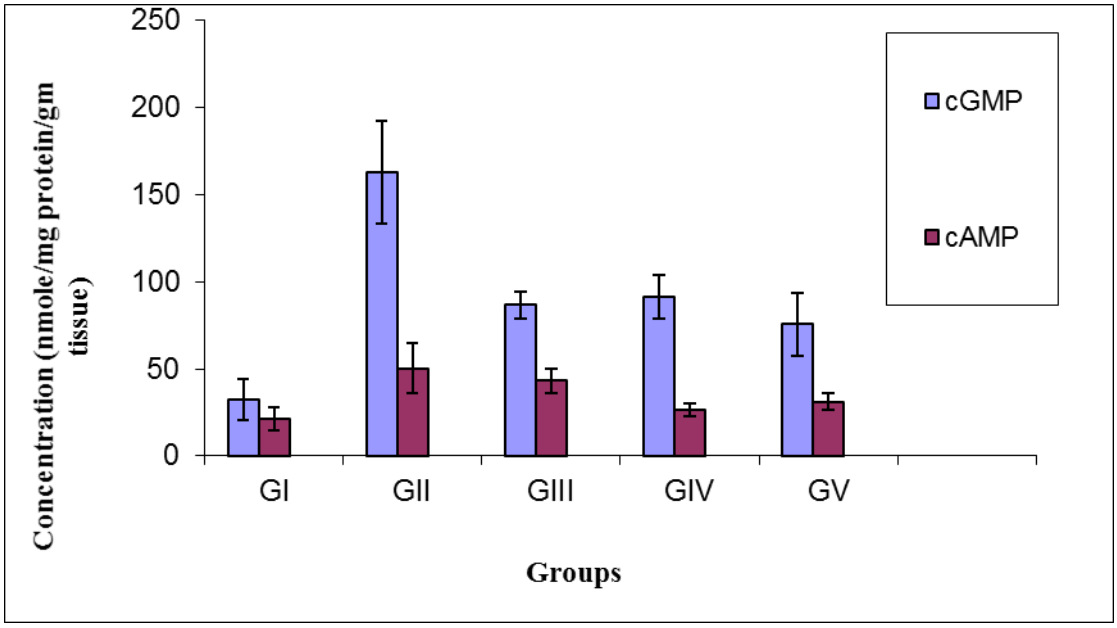

Figure 4: Brain cAMP and cGMP levels (Mean ± SE)

Discussion

Whey protein showed in suppression of nuclear factor kappa B (NF-κB) signaling, which is activated by TNF-α or the receptor activator of NF-κB ligand. The present study demonstrated that the interference with the overproduction of ROS by Whey protein + *Brassicaceae* extract in the $CdCl_2$ injected rats . Whey protein +

Brassicaceae extract reduced normalized downstream effectors of vascular response to injury. In addition, the reduction of free radicals overproduction suggest an indirect AGE-inhibiting effect of Combination treatment.
Our data suggested that part of the beneficial effect of Combination includes the disruption of the detrimental AGE-RAGE-NFκB pathways. It was found that whey protein effectively protect brain from $CdCl_2$-mediated protein glycation *in vitro*.Recent investigations suggested that *Brassicaceae* extract is an anti-inflammatory agent used frequently in Japanese traditional medicine, are also AGEs inhibitors [Ganong ,1997]. This is in accordance of our results which found that, Whey protein + *Brassicaceae* extract prevent the formation of AGEs and the inhibition neurodegenerations.

In the present study combined treatment with Whey protein + *Brassicaceae* extract have a good glycemic index control than individual one. This is reflected by increase body weight of treated diabetic rats compared with untreated.

The antioxidant capacity of combined treatment elevated significantly compared with untreated. This indicate the efficacy of these compounds in prevention of ROS production. The antioxidant property of whey protein [Hsu et al., 2006] and *Brassicaceae* extract [Kar and Mishra,1976] explained their role in improving antioxidant capacity and suppress ROS generation in diabetic rats [Lands etal.,1999 and Hamadeh,2000].

The elevation of cGMP level in $CdCl_2$ rats mediated the formation of AGEs was indicated its role in diabetic neuropathy. Combination lowered cGMP level but not cAMP level that indicate mediator specificity in its action.
The present study clearly demonstrates that controlling $CdCl_2$ and catalytic reactive oxygen scavenging are effective approach for the prevention of diabetic retinopathy. Combination whey protein + *Brassicaceae* extract treatment is a paradigm natural food supplement with a broad spectrum of beneficial biochemical and cell biological effects, based on its ability to reduce the $CdCl_2$ -induced ROS overproduction. Since Whey protein + *Brassicaceae* extract has beneficial effects on other target tissues of diabetic angiopathy, this concept appears attractive for the prevention or delay of diabetic angiopathy. In conclusion, it can be postulated that Whey protein + *Brassicaceae* extract could have potential benefits in the prevention of the onset and progression of neuropathy in diabetic patients.

Acknowledgment

This work was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant no. (RG-9-130-37). The authors, therefore, acknowledge with thanks DSR technical and financial support.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Agrawal RP, Beniwall R, Sharma S, Kochar DK, Tuteja FC, Ghorui SK and Sahan MS (2005). Effect of raw camel milk in type I diabeteus: One year randomized study. Journal of Camel Practice and Research 12(1): 27-35.
2. Agrawal RP, Swani SC, Beniwall R, Kochar DK,Sahani MS, Tuteja FC and Ghorui SK (2003) Effect of camel milk on glycaemic control, lipid profile and diabetes quality of life in type –1 diabetes: a randomised perspective controlled cross over study. India Journal of Animal Sciences 73 (10): 1105-1110.
3. Alberti KG and Zimmet PZ (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15: 539-553.
4. Al-Lawati JA, Al Riyami AM, Mohammed AJ, Jousilahti P (2002). Increasing prevalence of diabetes mellitus in Oman. Diabet Med 19: 954-957.
5. Ashour M, Salem S, Hassaneen H, EL-Gadban H, Elwan N, Awad A and Basu TK (1999). Antioxidant status and insulin dependent diabetes mellitus (IDDM). J Clin Biochem Nutr 26: 99-107.
6. Badr G, Ebaid H, Mohany H (2011) Modulation of immune cell proliferation and chemotaxis towards CC chemokine ligands (CCL)-21 and CXC chemokine ligand (CXCL)-12 in un-denatured whey protein-treated mice. *J nutrit Biochem*, In Press.
7. Beg OU, Von Bahr-Lind Strom H, Zaidi ZH and Jornvall H (1986). A camel milk protein rich in half cystine. Primary structure assessment of variations, internal repeat patterns and relationship with neurophysin and other active polypeptides. European Journal of Biochemistry 5(1): 195-201.
8. Fowler BA. Monitoring of human populations for early markers of cadmium toxicity; a review. Toxicol Appl Pharmacol 2009; 238: 294-300.
9. Satarug S, Garrett SH, Sens MA, Sens DA. Cadmium, environmental exposure and health outcomes. Environ Health Perspect 2010; 118: 182-190.
10. Renugadevi j, Miltonprabu S. Protective role of alpha tocopherol and ascorbic acid against cadmium induced neurotoxicity in rats. Int J Med Sci 2009; 2: 11-17.

doi:10.21010/ajtcam.v14i2.25
11. El-demerdash FM, Yousef MI, kedwany FS, Baghdadi HH. Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: Protective role of vitamin E and beta-carotene. Food Chem Toxicol 2004; 42: 1563-1571.
12. Ebaid H, Hassnein K and El-Feki M (2005). The un-denatured whey protein enhanced wound healing in mice. J Egyp Germ Soc 40(c): 2-27.
13. Ebaid H, Badr G, Metwalli A. (2011). Immunoenhancing property of dietary un-denatured whey protein derived from three camel breeds. Submitted to Nutrition Research.
14. Eeg-Olofsson K, Cederholm J, Nilsson PM (2008). Risk of cardiovascular disease and mortality in overweight and obese patients with type 2 diabetes: an observational study in 13,087 patients. Diabetologia 52: 65-73.
15. Floyd JC, Fajans SS, Pek S, Thiffault CA, Knopf RF, Conn JW (1970). Synergistic effect of certain amino acid pairs upon insulin secretion in man. Diabetes 19:102-8.
16. Ganong W (1997). Review of Medical Physiology, 18th ed. Stamford, CT: Appleton & Lange.
17. Hamadeh RR (2000). Noncommunicable diseases among the Bahraini population: a review. East Mediator Health J 6: 1091-1097.
18. Hsu WT, Tsai LY, Lin SK, Hsiao K and Chen BH (2006). Effects of diabetes duration and glycemic control on free radicals in children with type 1 diabetes mellitus. Ann Clin Lab Sci 36:174-8.
19. Joseph P, Murthy KR, Nelson JL and Kulkrani AP (1999). Peroxidase: a novel pathway for chemical oxidation in human term placenta. Placenta 13: 545-554.
20. Kar M and Mishra D (1976). Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiol 57: 315-319.
21. Lands LC, Grey VL and Smountas AA (1999). The effect of supplementation with A cysteine donor on muscular performance. J Appl Physiol 87: 1381-1385.